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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of:	)
Nicholas S. BODOR	)
Serial No.: 807,034	) Group Art Unit: 125
Filed: December 9, 1985	) Examiner: Roberts, E.
For : SOFT STEROIDS HAVING	)
ANTI-INFLAMMATORY ACTIVITY	)

DECLARATION

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

SIR :

I, Kazuyuki NAKAGAWA, of 774-1, Oomatsu, Kawauchi-cho, Tokushima-shi, Tokushima-ken, Japan, declare that

1) I graduated from Osaka University, Faculty of Pharmacy in March 1963. Since January 1964 up till the present, I have been in the employ of Otsuka Pharmaceutical Co., Ltd., assignee of the above-identified application, and have engaged in research works with respect to synthesis and development of various organic chemical and medical compounds in the research laboratory of this company. I had obtained a doctor degree in pharmacy from Osaka University in July 1981.

2) I am familiar with the subject matter disclosed in

the above-identified application as well as the disclosures in the references cited against the claims.

3) In order to demonstrate the difference between the invention of the above-identified application and the cited references, the following experiments were conducted under my general direction and supervision.

Experiment 1

The compounds of the cited references (i.e., Phillipps et al (1) - U.S. Patent 4,093,721 and Phillipps et al (2) - U.S. Patent 3,856,828) were tested for the effects on granulation tissue formation and on thymus weight caused by implantation of cotton pellets in rats in the same manner as described under the heading of GRANULOMA FORMATION TEST on page 38 of the Specification.

The compounds tested were the following three compounds, each having an alkanoyloxy group at the 17α-position.

Compound A

Chloromethyl 9α-fluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-dien-17β-carboxylate

(Compound of Example 12 of USP 3,856,828)

Compound B

Chloromethyl 9α-fluoro-11β-hydroxy-16β-methyl-3-oxo-17α-propionyloxyandrosta-1,4-dien-17β-carboxylate

(Compound of Example 1 or claim 11 of USP 3,856,828)

Compound C

Chloromethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-propionyloxyandrosta-1,4-dien-17β-carboxylate (Compound within a genus of claim 1 of USP 4,093,721)

The test results are shown in Table 1 below.

Table 1

Treatment	Dose µg/pellet	N	Body weight gain (g)	Dry granuloma wt. mg	Dry granuloma wt. Inhibition %	Thymus wt. mg	Thymus wt. Decrease %
None		10	25.8±1.1	77.9±6.9		487±22	
Compound A	0.3	8	29.6±2.4	69.6±7.0	10.7	441±23	
	1	8	31.9±2.9	54.5±4.9*	30.0	486±26	
	3	8	26.0±1.2	40.4±2.8***	48.1	450±31	
	10	8	25.0±1.3	30.1±2.2***	61.4	465±15	
	30	7	22.0±1.7	23.7±3.1***	69.6	416±17*	14.6
None		8	42.5±1.6			696±43	
Compound B	30	5	37.4±2.0			610±16	12.4
	100	5	34.2±2.8*			427±54**	38.6
	300	5	28.6±0.4***			170±11***	75.6
	1000	5	-8.4±2.6***			85±5***	87.8
	3000	5	-19.0±1.2***			70±9***	89.9

Table 1 (continued)

Treatment	Dose μg/pellet	N	Body weight gain (g)	Dry granuloma wt. mg	Dry granuloma wt. Inhibition %	Thymus wt. mg	Thymus wt. Decrease %
None		10	28.7±2.0	71.7±5.8		454±24	
Compound C	0.3	7	30.1±2.2	49.0±4.6*	31.7	472±22	
	1	7	31.3±2.5	29.3±4.4***	59.1	478±24	
	3	7	25.9±2.3	21.4±1.5***	70.2	445±12	
	10	7	24.1±2.1	15.1±1.8***	78.9	376±15	(17.2)

*, p<0.05, **, p<0.01, ***, p<0.001. (Mean±S.E.)

Experiment 2

The compounds listed below were tested for the effects on granulation tissue formation and on thymus weight caused by implantation of cotton pellet in rats in the same manner as described under the heading of GRANULOMA FORMATION TEST on page 38 of the Specification, and ED_{50}'s (for anti-granuloma effect), ED_{40}'s (for thymolysis), relative potencies and therapeutic indices, were determined from the test results obtained. For determining the relative potency, betamethasone 17-valerate was assigned a potency value of 100, and the potency of the test compounds are expressed relative thereto. The therapeutic indices were calculated according to the following equation.

$$\text{Therapeutic index} = \frac{\text{Relative potency for anti-granuloma}}{\text{Relative potency for thymolysis}}$$

Thus, a significant increase in the therapeutic index is indicative of significant anti-inflammatory effect relative to lower side effect.

The compounds tested were as follows.

(1) The compounds of the references

- Compound A as used in Experiment 1
- Compound B as used in Experiment 2

(2) The compounds of the present invention

The claimed compounds tested were those which have ethoxycarbonyloxy group at the 17α-position and which have the same substituents as the Compound A or B at other

positions.

- Compound of Example 7A-3, i.e., chloromethyl 17α-ethoxycarbonyloxy-9α-fluoro-11β-hydroxy-16α-methylandrosta-1,4-dien-3-one-17β-carboxylate
- Compound of Example 7A-2, i.e., chloromethyl 17α-ethoxycarbonyloxy-9α-fluoro-11β-hydroxy-16β-methylandrosta-1,4-dien-3-one-17β-carboxylate

The ED_{50}'s, ED_{40}'s, relative polecies and therapeutic indices obtained were shown in Table 2 below.

Table 2

Test Compound	Anti-granuloma		Thymolysis		Therapeutic Index
	ED_{50} (µg/pellet)	Relative potency	ED_{40} (µg/pellet)	Relative Potency	
Compound of Ex.7A-3	1.07	7925	14700	1.44	5503
Compound A	4.04	2099	2090	10	210
Compound of Ex.7A-2	6.45	1315	>1000	<21	>62.6
Compound B	5.98	1418	89.0	238	6.0
Betamethasone 17-valerate	84.8	100	212	100	1

Consideration on the results of the experiments

As seen from Table 1, the compounds of the references cause significant decrease (12.4-17.2%) in thymus weight at a dose of as low as 10 or 30 μg/pellet.

As seen from Table 2, replacement of alkanoyloxy group at the 17α-position of the reference compounds with alkoxycarbonyloxy group (or carbonate ester group) causes remarkable increase in the therapeutic index. That is to say, the therapeutic index of compound of Example 7A-3 is about 26 times as high as that of Compound A, and the therapeutic index of the compound of Example 7A-2 is at least 10 times as high as that of Compound B.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 27, 1986

Kazuyuki Nakagawa

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